Intralesional Epidermal Growth Factor for Diabetic Foot Ulcers

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ABSTRACT

Objective: To determine the role of the intralesional recombinant epidermal growth factor (rEGF) in the healing and prevention of extremity amputation in advanced diabetic foot ulcer patients.

Study Design: Observational study.

Place and Duration of Study: Department of Cardiovascular Surgery, Duzce State Hospital, Duzce, Turkey, between November 2018 and September 2019.

Methodology: A total of 58 patients with diabetic foot ulcers that were treated at the study place were enrolled. The lesions were graded with Wagner Classification System. EGF (75 microg of Heberprot-P) vials were stored at +4°C and cold-chain requirements were followed. EGF 5 mL was dissolved with 0.09% saline solution; and 0.5-1 ml of the solution was injected into the tissues and edge of the lesions regularly. The data was evaluated at the end of two years of the treatment period. The primary objective was wound healing, formation of granulation tissue; and the secondary objective was the prevention of lower extremity amputation.

Results: Diabetic foot ulcers wound healing was achieved in 93.1% (n=54) of patients with the formation of granulation tissue. The complete recovery was observed in 94.1% (n=32) of the patients who had Grade III and IV lesions. Lower extremity amputation was performed in two (3.4%) subjects. The lesions of two patients required flap surgery. The most common adverse events were tremor and syncope.

Conclusion: Recombinant epidermal growth factor is highly effective for the treatment of diabetic foot ulcers and prevention of extremity amputation. Intralesional rEGf provides efficient and safe wound healing/closure in patients with diabetic foot ulcers.

Key Words: *Amputation, Epidermal growth factor, Diabetic foot, Wound healing.*

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INTRODUCTION

Topical and intralesional forms of rhEGF are widely used in the treatment of DFUs. Despite the appropriate medical treatment (*antibiotics, surgery, and hyperbaric oxygen)*, wound dressing and debridement, diabetes mellitus (DM) is still one of the leading causes of non-traumatic lower extremity amputation.[1]

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Diabetic foot ulcers are an important cause of non-traumatic lower extremity amputations. It is estimated that 19–34% of individuals with diabetes mellitus are likely to develop DFU in the rest of their life. Lower extremity infections are the most common reason for hospitalisation in patients with diabetes mellitus. The cure with standard care is possible only in 45% of DFU patients.[1,2] In previous research, it was emphasised that patients with diabetic foot pathologies fear major lower-extremity amputation more than death, foot infection, or end-stage renal disease.[3] In a systematic review, the annual extremity amputation rate was reported as 5% and five-year mortality rates for ischemic and neuropathic ulcers were 55% and 45%, respectively.[4]

Glycation process and advanced glycation end products *(AGEs)* are the main reasons in the development of various diseases

and their complications such as Type II DM, cardio-vascular disorders, osteoporosis and arthritis.[5,6] Glycation interferes with the major functions of regulatory enzymes and membrane systems and it is believed that AGEs have a negative impact on metabolic processes. AGEs lead to systemic inflammation and oxidative stress *via* binding AGE receptors expressed by inflammatory cells. They activate the release of interleukin-1, interleukin-6, tumour necrosis factor α (TNF-α) and insulin-like growth-factor-1 (IGF-1*)* by monocytes and macrophages. Several adhesion molecules, such as vascular cell adhesion molecule -1 (VCAM-1) and intracellular adhesion molecule, 1 (ICAM-1) that are synthesised by endothelial cells lead to endothelial inflammation. AGEs also have a role in nitrosative stress *via* the activation of the inducible nitric oxide synthase (NOS). These mediators cause an increase in insulin resistance and exacerbation of hyperglycemia which leads to a vicious cycle.[7,8]

The wound-healing effect of the EGF has inflammatory, fibroblastic and repair phases. As a mitogenic peptide, EGF starts to show its effect at the end of the inflammatory phase and induces the fibroblast formation after the stimulation of granulation tissue formation and epithelisation. EGF also initiates the DNS synthesis and cell production and activates protein synthesis.[9]

The rhEGF treatment has first been approved in Cuba in 2006. Studies showed that rhEGF facilitates the wound healing and accelerates the regulation of mRNA in newly formed tissues; and it reduces the hypoxia, inflammation and oxidative stress in DFUs and mediates the genetic alterations which induce cell proliferation, collagen synthesis and re-formation of the extracellular matrix and 80% of granulation tissue formation was demonstrated in clinical studies.[10,11]

Current evidence shows a decrease in growth factors, particularly in EGF and in diabetic foot wounds, one should bear in mind that EGF also has an angiogenic effect which supports the growth of the vascular network in the lesions.[12]

The objective of the present study was to evaluate the role of the intralesional recombinant epidermal growth factor in the healing and the prevention of extremity amputation in advanced diabetic foot ulcer patients. The authors have evaluated the efficacy and safety of the intralesional rhEGF treatment in DFU healing and the prevention of LEA (lower extremity amputation) and presented the data from the largest single--centre patient population group in Turkey.

METHODOLOGY

The current research was conducted as a multidisciplinary approach in the Department of Cardiovascular Surgery, Plastic and Reconstructive Surgery, Infectious Diseases and Clinical Microbiology of Duzce State Hospital, Turkey. A total of 58 individuals with Type II diabetes mellitus (T2DM), that have been admitted to the clinic between November 2018 to September 2019, have been enrolled in the analysis.

The study population consisted of subjects who have been administered intralesional recombinant epidermal growth factor (rhEGF). Patients with cancer, who have a life expectancy of less than one year, dermal and subdermal infections, osteomyelitis, renal failure and hepatic failure; patients receiving corticosteroids and immunosuppressive agents and patients who had Wagner Grade V CFUs were excluded from the study. The lesions were graded with Wagner Classification System.

Patients were monitored by cardiovascular surgery, infectious diseases and clinical microbiology departments. Endocrinology clinic followed the patients for the regulation of blood glucose levels.

EGF (75 microg of Heberprot-P) vials were stored at +4°C and cold-chain requirements were followed. EGF 5 mL was dissolved with 0.09% saline solution and 0.5-1 ml of the solution was injected into the tissues and edge of the lesions regularly. The clinical improvement was monitored.

Ampicillin-sulbactam was given to the patients who had signs of wound infection. The samples were taken from lesions for cultivation and antibiotic treatments were applied according to test results. Concomitant antibiotics and intralesional EGF were administered throughout the course of treatment in patients who require antibiotic cure. Intralesional EGF (Heberprot b 75 µg) injection has been conducted to the edges of the lesions from 1 to 8 weeks (2-3 times per week). The sterile wound dressing has been practised after each injection and appropriate sterile debridement has been carried out, when necessary.

The size of the lesions was recorded at the first admission and after the treatment period. The ulceration area was recorded as cm^2 digitally with Scion Image (NIH Image, Version 1.52; National Institutes of Health; Bethesda, MD) software.

The enrolled patients have been investigated *via* doppler USG, CT and MR angio. Individuals have also received medical treatment before the injection of intralesional rhEGF. The logic behind this initial treatment can be elaborated as utilising all possible options and position intralsional rhEGF in uncured diabetic ulcer cases.

The study results were concluded after the evaluation of two-year data retrospectively. The healing criteria has been designated as the formation of the granulation tissue and the reduction in the size of the lesion area prior to and after the injection.

All the patients were informed about the procedure in detail and provided written informed consent at enrollment. The study protocol was reviewed and approved by the Institutional Ethics Committee (17.09.2018-2018/174-Duzce University).

The data was collected and analysed with IBM software SPSS version 22. The qualitative data were given as numbers and proportions, while quantitative as mean ± SD and median (IQR: 25th percentile-75th percentile). Wilcoxon sign rank test was used to compare wound sizes before and after the procedure. A p-value of <0.05 is considered as statistically significant.

RESULTS

The mean age of the study population was 66.12 ± 5.55 years with a median disease duration of 15 (13-19) years. Gender distribution was quite even as 43.1% (n=25) of the subjects were male. Diabetic foot ulcerations were present approximately for 4 (3-8) months. Peripheric vascular disease was the most prominent disorder as 62.1% (n=36) in the initial phase. Renal failure has been observed in 10.3% (n=6), infection in 10.3% (n=6) and previous balloon angioplasty, by-pass (peripheral) in 15.5% (n=9) of the cases. Baseline characteristics of patients are summarised in Table I.

Table I: Baseline characteristics of the study population and efficacy results characteristics.

Age (years) mean ± SD	66.12±5.55
Gender (male)	25 (43.1%)
Duration of diabetes (years), (median)	15 (13-19)
Duration DFU (months), (median)	4 (3-8)
Renal failure	6 (10.3%)
PVD	36 (62.1%)
Balloon angioplasty, by-pass (periferik)	9 15.5%)
Infection	6 (10.3%)
Outcome	
Pre wound size (cm^2), (median), (IQR)	15.2 (12.1-18.4)
Post wound size (cm^2), (median), (IQR)	3.3 (2.2-4.6)
End of follow period (months)	24
Outcome	
Complete healing (>75%)	54 (93.1)
Complete healing (Wagner IIIveIV)	32/34 (94.1%)
DFU: Diabetic foot ulcer, PVD: Peripheral vascular disease.	

Table II: Bacteria isolated from diabetic ulcers.

Agent	**N%**
Gram-positive cocci	4
Staphylococcus aureus	4
Methicillin sensitive	2
Methicillin-resistant	2
Gram-negative bacilli	6
Pseudomonas aeruginosa	2
Proteus spp.	1
P. mirabilis	1
Klebsiella pneumoniae	2
Total	10 (100%)

Complete healing was observed with rhEGF in 32 patients with Grade III or IV lesions (94.1%). Only 2 out of 34 patients with Wagner Grade III or IV underwent LEA (5.9%). Both patients had chronic renal failure. The lesions of 2 patients with Grade III ve IV were treated with Flap Surgery. Recurrence of the lesions was observed in 2 patients. The decrease in the size of the lesion between pre- (wound size cm^2=15.2) and post-application (wound size cm^2=3.3) was statistically significant ($p<0.001$).

Different microorganisms were isolated from the lesions *via* cultuvation. Two patients had infection with pseudomonas aeruginosa. Despite the appropriate treatment given, one patient has undergone LEA (Table II).

Diabetic foot ulcers wound healing was achieved in 93.1% (n=54) of patients with the formation of granulation tissue. The complete recovery was observed in 94.1% (n=32) of the patients who had Grade III and IV lesions. Lower extremity amputation was performed in two (3.4%) subjects. The lesions of two patients required flap surgery. The most common adverse events were tremor and syncope.

The most common adverse events during intralesional EGF application were tremor, near syncope, pain and rash.

DISCUSSION

Effective metabolic control in diabetic patients plays a key role in the treatment of DFU. Revascularisation, balloon angioplasty, stent and by-pass surgery are inevitable treatment modalities with the development of complications. Despite advancements in these treatment options, secondary treatment modalities like infection control, wound dressing/debridement, hyperbaric oxygen/ozone therapy and negative pressure wound therapies should be utilised in necessary cases. RhEGF has shown in successful outcomes as an expensive treatment option.

It is unfortunate that limited number of high-quality studies have been published in wound care up-to-date. Most studies have an insufficient number of patients, short follow-up periods and not well-designed control groups. In a randomised, double-blind, controlled study 149 patients with Wagner III ve IV lesions were randomised into EGF (8 weeks – 3 weeks, 75 or 25µg) and placebo arms. The 83% granulation formation has been demonstrated at week two in patients who received EGF 75µg, which was 39% in the control arm. The complete response was 52% in the control arm; whereas, it was 52% and 75% in patients who received 25µg and 75µg, respectively in the treatment arm at week 5.[13] The prevention rate of the LEA was reported as 58.6% in a clinical study conducted in 2006.[14] In a review, which summarised the available clinical information of 2000 patients who received intralesional rhEGF for advanced DFU showed a 71% relative reduction of the risk of amputation.[15]

There were a considerably high number of studies evaluating the DFUs and its treatment with rhEGF. The common results of these studies could be stated as the positive effect of rhEGF on tissue healing, reducing the recurrence of chronic refractory DFUs and preventing LEA. Despite all these unaddressed situa-

tions, there is no consensus on the dosage and the treatment duration of EGF. The results of previous research has designated that granulation formation and the closure of the ulcer were achieved in early weeks of the treatment and healing with 75µg (2 to 8 weeks; 2-3 per week).[13,15,16]

Despite the positive results and recommendations of current clinical data, (considering the short duration of patient follow-up – several months), better outcomes should be supported by the long-term efficacy and safety results of rhEGF for a better understanding.

It was demonstrated that the frequency of amputation increased with the thickening of the ulcer and advanced stage, leading to amputation risk (11 times in ulcers progressing to the bone and 90 times in cases with co-infection and ischemia). In addition, it has been reported that Wagner Staging is an independent risk factor.[17]

Existing ulcers should be treated aggressively at an early stage to prevent DFUs. The aim of the clinician should be eradicating the occurrence of the advanced stage after repeated mechanical stress, thermal or mechanical injury leading to reduced incidence of amputation. Inefficient fasting blood glucose regulation, lipid control, renal failure, cardiac complications, ischemic and neuropathic peripheral vascular disease complications derive the medical team to work with a multidisciplinary approach.

Standard topical rhEGF may help heal DFUs during the first weeks of treatment. While topical administration can heal ulcers with Wagner Grade II or III DFUs, intralesional rhEGF injection remains a good treatment option for more severe ulcers. The epidermal growth factor is more effective in Wagner III and IV DFUs. Lower extremity amputation rate is higher in these patients because complex clinical and pathological features of DFUs make wound healing difficult with standard therapy options.[18]

In this study, granulation formation and wound healing were achieved at a significant rate (94.1%) in patients with Wagner III and IV DFUs. Thus, III and IV patients with a high amputation rate were not amputated, and significant quality of life improvement has been observed (Figures 1 and 2).

Although the application of intralesional EGF to the wound edges is easy and practical, it is quite an expensive treatment method. Considering the costs related with diabetes and its complications, the authors believe that intralesional EGF should be a preferred treatment approach for DFUs as it prevents relapses, reduces extremity amputations and most importantly improves the quality of life of patients.

In addition, the intralesional application has an impact in deeper layers compared to topical application. Patients can easily tolerate mild side effects during application. Intralesional EGF treatment should not be considered as an alternative to standard revascularisation or debridement and antibiotherapy. After the occurrence revascularisation in diabetic patients, intralesional EGF application should be performed together with metabolic control, antibiotherapy and wound care/debridement.

The relatively low number of patients may be considered as a limiting factor in this study. Another item can be referred to the study design as it was not randomised and prospective. On the other hand, the current study was the largest single-centre patient population group in Turkey.



Figure 1: (A) Grade 4 DFU at admission, (B) Post rfEGF application, week 4, (C) Complete closure of Grade 4 DFU at week 8.

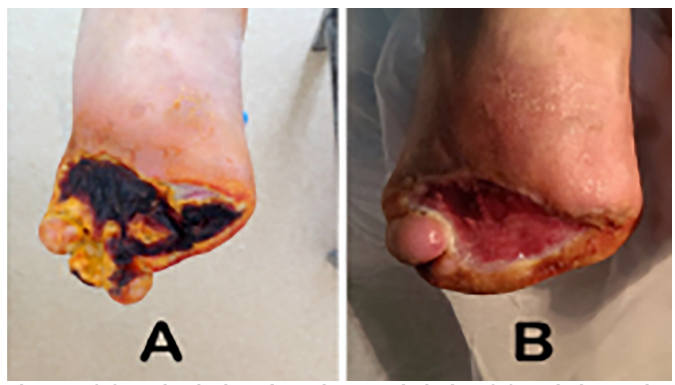


Figure 2: (A) Grade 4 lesion of a patient at admission, (B) Healed wound at week 8.

CONCLUSION

The recombinant epidermal growth factor is highly effective for the treatment of diabetic foot ulcers and prevention of extremity amputation. Intralesional recombinant epidermal growth factor provides efficient and safe wound healing/closure in patients with diabetic foot ulcers.

ETHICAL APPROVAL:

The study protocol was reviewed and approved by the Institutional Ethics Committee at Duzce University.

PATIENTS' CONSENT:

All participants signed the informed consent before enrollment in the study.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

EI: Collected and analysed data, wrote the manuscript.
FBGD: Collected data.
ACD: Analysed data.
HA: Searched literature.
AU: Designed study, agreed to be accountable for all aspects of the work.
MK: Developed the theoretical framework.

REFERENCES

1. Rorive M, Scheen AJ. News in the management of diabetic foot. *Rev Med Suisse* 2019; **15(659)**:1448-52. PMID: 3143 6060.
2. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med* 2017; **376(24)**:2367-75. doi:10.1056/NEJMra1615439.
3. Wukich DK, Raspovic KM, Suder NC. Patients with diabetic foot disease fear major lower-extremity amputation more than death. *Foot Ankle Spec* 2018; **11(1)**:17-21. doi:10. 1177/1938640017694722.
4. Jupiter DC, Thorud JC, Buckley CJ, Shibuya N. The impact of foot ulceration and amputation on mortality in diabetic patients. I: From ulceration to death, a systematic review. *Int Wound J* 2016; **13(5)**:892-903. doi:10.1111/iwj.12404.
5. Sanguineti R, Puddu A, Mach F, Montecucco F, Viviani GL. Advanced glycation end products play adverse proinflammatory activities in osteoporosis. *Mediators Inflamm* 2014; **2014**:975872. doi:10.1155/2014/975872.
6. Vlassara H, Bucala R. Recent progress in advanced glycation and diabetic vascular disease: Role of advanced glycation end product receptors. *Diabetes* 1996; **45(Suppl 3)**:S65-66. doi:10.2337/diab.45.3.s65.
7. Chuah YK, Basir R, Talib H, Tie TH, Nordin N. Receptor for advanced glycation end products and its involvement in inflammatory diseases. *Int J Inflam* 2013; **2013**:403460. doi:10.1155/2013/403460.
8. Ott C, Jacobs K, Haucke E, Navarrete Santos A, Grune T, Simm A. Role of advanced glycation end products in cellular signaling. *Redox Biol* 2014; **2**:411-29. doi:10. 1016/j.redox.2013.12.016.
9. Tiaka EK, Papanas N, Manolakis AC, Georgiadis GS. Epidermal growth factor in the treatment of diabetic foot ulcers: An update. *Perspect Vasc Surg Endovasc Ther* 2012; **24(1)**:37-44. doi:10.1177/1531003512442093.
10. Camacho-Rodríguez H, Guillen-Pérez IA, Roca-Campaña J, Baldomero-Hernández JE, Tuero-Iglesias ÁD, Galván-Cabrera JA, *et al.* Heberprot-P's effect on gene expression in healing diabetic foot ulcers. *MEDICC Rev* 2018; **20(3)**:10-4. doi: 10. 37757/MR2018.V20.N3.4.
11. Zhang J, Hu W, Diao Q, Wang Z, Miao J, Chen X, *et al.* Therapeutic effect of the epidermal growth factor on diabetic foot ulcer and the underlying mechanisms. *Exp Ther Med* 2019; **17(3)**:1643-8. doi:10.3892/etm.2018.7133.
12. Garcia Herrera AL. Intralesional administration of human recombinant epidermal growth factor improves healing and reduces amputations in patients with severe diabetic foot ulcers. *Curr Ther Res Clin Exp* 2017; **85**:29-30. doi:10. 1016/j.curtheres.2017.03.004.
13. Fernández-Montequín JI, Valenzuela-Silva CM, Díaz OG, Savigne W, Sancho-Soutelo N, Rivero-Fernández F, *et al.* Intra-lesional injections of recombinant human epidermal growth factor promote granulation and healing in advanced diabetic foot ulcers: multicenter, randomised, placebo-controlled, double-blind study. *Int Wound J* 2009; **6(6)**:432-43. doi:10.1111/j.1742-481X.2009.00641.x.
14. Acosta JB, Savigne W, Valdez C, Franco N, Alba JS, del Rio A, *et al*. Epidermal growth f0actor intralesional infiltrations can prevent amputation in patients with advanced diabetic foot wounds. *Int Wound J* 2006; **3(3)**:232-9. doi:10.1111/j. 1742-481X.2006.00237.x.
15. López-Saura PA, Yera-Alos I, Carmen VS, Odalys GD, Amaurys del RM, Jorge BA, *et al.* Medical practice confirms clinical trial results of the use of intralesional human recombinant epidermal growth factor in advanced diabetic foot ulcers. *Advances in Pharmacoepidemiology Drug Safety* 2013; **02**:128. doi:10.4172/2167-1052.1000128.
16. Xu J, Min D, Guo G, Liao X, Fu Z. Experimental study of epidermal growth factor and acidic fibroblast growth factor in the treatment of diabetic foot wounds. *Exp Ther Med* 2018; **15(6)**:5365-70. doi:10.3892/etm.2018.6131.
17. New JP, McDowell D, Burns E, Young RJ. Problem of amputations in patients with newly diagnosed diabetes mellitus. *Diabet Med* 1998; **15(9)**:760-4. doi: 10.1002/(SICI)1096-9136(199809)15:9<760::AID-DIA672>3.0.CO;2-I.
18. Viswanathan V, Juttada U, Babu M. Efficacy of Recombinant human epidermal growth factor (Regen-D 150) in healing diabetic foot ulcers: A hospital-based randomised controlled trial. *Int J Low Extrem Wounds* 2020; **19(2)**: 158-64. doi: 10.1177/1534734619892791.